cytokines (including IL-2, IL-4, γ-IFN and TNF-α) and an early activation antigen (such as CD69), in combination with one or more T cell subset-defining phenotypic markers (such as CD3 or CD4). It is further disclosed that the antigen specific response can be more easily detected when the antigen stimulation is provided in conjunction with costimulation of surface antigens involved with accessory cell surface molecules, such as CD28, CD40, VLA-4, and other such specificities known in the art. Costimuli can be either antibodies or ligand binding to these antigens. A preferred costimulus is CD28. It is also determined that T cell responses measured using the protocol defined in this disclosure can be shown to be sensitive to drugs which have been demonstrated to augment or suppress cellular responses (e.g., exogenous cytokines, cyclosporine-A, herbimycin-A).

IN THE CLAIMS:

Please cancel claims 56 - 60 without prejudice.

Please amend claim 19 by rewriting the claim to read as follows.

19 (once amended). A method of detecting antigen-specific T lymphocytes, comprising:

contacting a sample containing peripheral blood mononuclear cells with an MHC-dependent nominal antigen;

adding to said sample an inhibitor of cytokine secretion;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and then

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

Please add the following new claims:

61 (new). The method of claim 19, wherein each of said at least one cytokine-specific antibody is specific for a cytokine selected from the group consisting of IL-2, IL-4, IL-13, IFN-γ, and TNF-α.

62 (new). The method of claim 61, further comprising the step of adding to said sample, contemporaneously with antigen contact, a costimulus of T cell activation, wherein said costimulus is selected from the group consisting of antibodies specific for CD28, VLA-4, CD86, or CD118.

63 (new). The method of claim 61, further comprising contacting said sample with an antibody specific for CD69, and then flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by $CD69^+$ cells in the defined T lymphocyte subset.

REMARKS

Status of the claims

Claims 19 - 60 were pending. Pursuant to restriction requirement, claims 56 - 60 were withdrawn as directed to independent and distinct inventions. Claims 56 - 60 have now been cancelled in favor of the filing of